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Sensitive Detection Technique of Myeloperoxidase Precursor Protein by Flow Cytometry With Monoclonal Antibodies

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This report describes the analysis of culture cells and blast cells separated from the heparinized bone marrow and whole blood of patients with acute leukemias by means of a density-gradient technique (Ficoll-sodium metrizoate d = 1.077 g/cm^3). Cell-surface antigens were analyzed by a fluorescence-activated cell sorter using a panel of monoclonal antibodies (MAbs). The blast cells and culture cells were fixed by 3% paraformaldehyde in phosphate-buffered saline. A low level of expression of MPO precursor protein was found in THP−1. K-562 and HEL, MEG-01, erythro-megakaryocytic leukemia cell lines, Jurkat, MOLT-3, MOLT-4, RPMI8402, ATL-5, T-cell leukemia cell lines, Raji, Daudi. BALL-1, B-cell leukemia cell lines, and AGNK1 showed negative reaction. The de novo MPO-negative acute leukemias, middle level of expression of MPO precursor protein, was found in the blasts of MPO-negative AML (AML, M0), which coexpressed CD13, CD33, CD34, and CD38. A high level of expression of MPO protein was found in all cases of AML, M1, and M2. The MPO expression was not found in all cases of acute lymphoblastic leukemia. The highest level of MPO expression was found in cases of AML, M3, and AML, M3v, suggesting the diagnostic value for this type of leukemia. The detection of MPO precursor protein by flow cytometric analysis with monoclonal antibodies is essential for the determination of lineage and precise diagnosis of acute unclassifiable leukemia, and should contribute substantially to the development of an effective form of therapy and cure. Am. J. Hematol. 58:241–243, 1998. © 1998 Wiley-Liss, Inc.

Key words: MPO precursor protein; flow cytometric analysis; monoclonal antibodies; MPO gene

INTRODUCTION

The detection of MPO precursor protein by flow cytometric analysis with monoclonal antibodies (MAbs) is essential for the determination of lineage and precise diagnosis of acute unclassifiable leukemia. Myeloperoxidase (MPO) cDNA was originally cloned by Morishita et al. [1] who found a single MPO gene with 20,235 nucleotides coding for a protein of 745 amino acids with a calculated molecular weight (MW) of 83,868 daltons. It is generally accepted that MPO is primarily synthesized as a precursor protein with an MW of 89,000–91,000, which subsequently cleaves into the heavy and the light chains to yield the mature active protein. Recently, Serrano et al. [2] reported that blast cells of the patients with acute lymphoblastic leukemia expressed significant levels of MPO gene (MPO mRNA) by reverse transcription polymerase chain reaction (RT-PCR) without the translation to MPO protein. We describe here the highly sensitive technique for detection of the MPO precursor protein by means of flow cytometric analysis with MAbs, MA1, and A2-3 [3]. This technique was primarily established on the same line for detection of minimal residual disease in acute lymphoblastic leukemia [4].

MATERIALS AND METHODS

Cell Separation

Mononuclear cells (PBMC and BMMC) were isolated from heparinized peripheral blood and bone marrow by a

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density-gradient technique (Ficoll-sodium metrizoate d = 1.077 g/cm^3). They then were washed twice with phosphate-buffered saline (PBS) medium and resuspended in the same medium. Subsequently, the cells were used for flow cytometric analysis as described below.

Cell Culture

Cells used in this study were: HL60, human promyelocytic leukemia cell line; THP-1, human monoblastic leukemia cell line; K-562 and HEL, erythroleukemia cell lines; MEG-01; megakaryoblastic leukemia cell line [5], MOLT-3, MOLT-4, RPMI8402, and ATL-5 [6]; T-cell leukemia cell lines, Raji, Daudi, and BALL-1; B-cell leukemia cell lines (provided by Drs. J. Minowada and K. Orita, Fujisaki Cell Center, Okayama, Japan); AGNK1, natural killer cell leukemia cell line [7]; Jurkat, a human T-cell leukemia cell line (obtained from Japanese Cancer Research Resources Bank, Tokyo, Japan). All cells were maintained in continuous culture in RPMI 1640 culture medium (Grand Island Biochemical Co., Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Microbiological Associates, Bethesda, MD), penicillin G (200 U/ml), streptomycin (50 μg/ml), 2 mM L-glutamine, and sodium bicarbonate.

Monoclonal Antibodies

Monoclonal antibodies (MAbs) were obtained either commercially from Ortho Diagnostic Systems K.K. (Tokyo, Japan), Japan Coulter Co. (Tokyo, Japan), Nichirei Co. (Tokyo, Japan), Becton Dickinson (Tokyo, Japan) and Dako Co. (Kyoto, Japan), or through the courtesy of Dr. Morishita (Nagoya University, Nagoya, Japan).

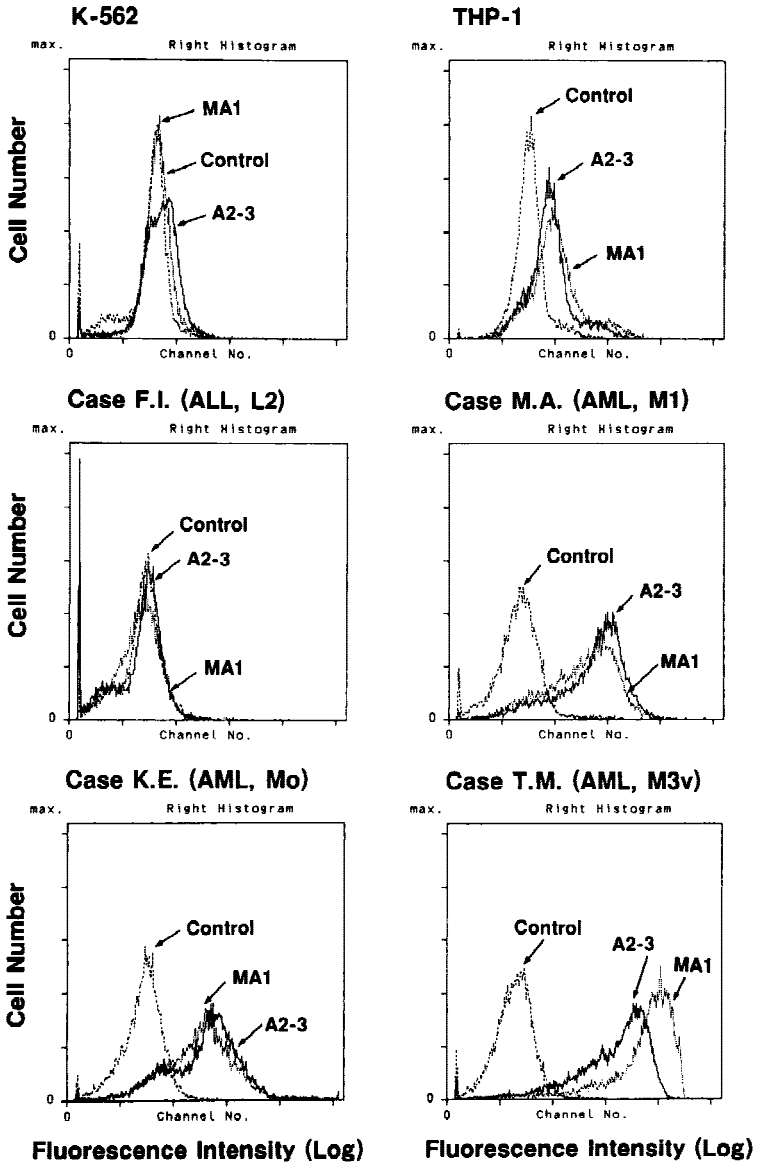

Fig. 1. Flow cytometric analysis of the expression of myeloperoxidase precursor protein by the use of anti-MPO monoclonal antibodies, MA1 and A2-3 clones, which recognize the epitopes of MPO precursor protein. Most of the cell lines were provided by Drs. J. Minowada and K. Orita (Fujisaki Cell Center, Okayama, Japan).

Indirect Immunofluorescence Testing

Cells (5×10^5) were incubated with 10μl of MAbs for 45 min at 4°C, washed twice with PBS, and then incubated with a fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated $F(ab')_2$ fragment of goat anti-mouse immunoglobulin (Ig) reactive with gamma or mu (Biosource Int., Camarillo, CA) for 30 min in 4°C. After incubation, the cells were washed twice and analysed by flow cytometry. Direct labelled (FITC or PE) MAbs were used for the two-color analysis.

Staining for MPO Precursor Protein

Cells were fixed in 1 ml of a solution containing 3% paraformaldehyde in PBS for 10 min at 4°C, washed twice with PBS, and incubated with anti-MPO MAbs, MA-1, and A2-3 (unpublished MAb), which react with MPO precursor protein. After two washes with PBS, FITC-goat anti-mouse IgG (10 μl) was added for 30 min at room temperature, and two additional washes with PBS were performed prior to fluorescence-activated cell sorter (FACS IV and FACS Vantage) analysis, as described previously [7,8]. More than 200,000 cells were identified for the two-color analysis.

RESULTS

A low level of expression of MPO precursor protein was found in THP-1, whereas K-562 and HEL, MEG-01, Jurkat, MOLT-3, MOLT-4, RPMI8402 and ATL-5, T-cell leukemia cell lines, Raji, Daudi, and BALL-1, and AGNK1 showed negative reaction (Fig. 1).

For the de novo MPO-negative acute leukemias, middle level of expression of MPO precursor protein was found in the blasts of MPO-negative AML (AML, M0), which expressed CD13, CD33, CD34, and CD38 as previously described [3,8]. The blasts did not possess lymphoid antigens, CD19, cytoplasmic CD22, CD79a, and cytoplasmic CD3. A high level of expression of MPO protein was definitely found in all cases of AML, M1, and M2, whereas the MPO expression was not found in all cases of acute lymphoblastic leukemia (Fig. 1). The highest level of MPO expression was found in cases of AML, M3, as well as AML, M3v (Fig. 1), suggesting the diagnostic value for this type of leukemia.

DISCUSSION

The low levels of expression of MPO precursor protein that were found in THP-1 cell by flow cytometric analysis could not be observed by means of indirect immunofluorescence test and immunocytochemistry. This indicates a higher sensitivity for flow cytometric analysis. The RT-PCR method is known as the most sensitive assay and useful adjunct when it is carried out stringently. However, it should be recognized that peripheral blood in acute lymphoblastic leukemia contains a few numbers of both normal myeloid MPO-negative and MPO-positive precursors, especially among childhood leukemia patients. In addition, MPO mRNA sequence has high homologies with eosinophil peroxidase [9] as well as recently cloned lactoperoxidase [10], which locate to adjacent sites of MPO genes in chromosome 17q21-q24. The possible detection of pseudogenes in the leukemic blasts should also be noted since we have no reports demonstrating the expression of MPO genes in the blasts of lymphocytic leukemias as well as lymphoid cell lines.

In summary, our data show that the detection of MPO precursor protein by flow cytometric analysis with monoclonal antibodies is essential for the determination of lineage and precise diagnosis of acute unclassifiable leukemia, and should contribute substantially to the development of an effective form of therapy for its cure.

REFERENCES

1. Morishita K, Kubota N, Asano S, Kaziro Y, Nakata S: Molecular cloning and characterization of cDNA for human myeloperoxidase. J Biol Chem 262:3844–3851, 1987.
2. Serrano J, Roman J, Sanchez J, Torres A: Myeloperoxidase gene expression in acute lymphoblastic leukaemia. Br J Haematol 97:841–843, 1989.
3. Imamura N, Kuramoto A: Acute unclassifiable leukaemia in adults, demonstrating myeloid antigens and myeloperoxidase protein. Br J Haematol 69:427–428, 1988.
4. Imamura N, Kuramoto A: Detection of minimal residual disease in acute lymphoblastic leukemia by flow cytometry with monoclonal antibodies. Am J Hematol 38:332–334, 1991.
5. Imamura N, Mtasiwa DM, Inada T, Kuramoto A: Different expression of CD36 antigen molecule on the surface of megakaryocyte lineage leukemias and megakaryocyte leukemia cell lines MEG-01 and HEL. Leukemia 4:525–528, 1990.
6. Imamura N, Inada T, Tagaya Y, Yodoi J, Kuramoto A: Association between ATL and non-hematopoietic neoplasms. Hematol Oncol 11:127–137, 1993.
7. Imamura N, Kusunoki Y, Kawa-Ha K, Yumura K, Hara J, Oda K, Abe K, Dohy H, Inada T, Kajihara H, Kuramoto A: Aggressive natural killer cell leukaemia/lymphoma; Report of four cases and review of the literature. Possible existence of a new clinical entity originating from the third lineage of lymphoid cells. Br J Haematol 75:49–59, 1990.
8. Imamura N, Tanaka R, Kajihara H, Kuramoto A: Analysis of peroxidase negative acute unclassifiable leukemias by monoclonal antibodies. I. Acute myelogenous leukemia and acute myelomonocytic leukemia. Eur J Haematol 41:420–428, 1988.
9. Sakamaki K, Tomonaga M, Tsukui K, Nagata S: Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase. J Biol Chem 264:16828–16836, 1989.
10. Ueda T, Sakamaki K, Kuroki T, Yano I, Nagata S: Molecular cloning and characterization of the chromosomal gene for human lactoperoxidase. Eur J Biochem 243:32–41, 1997.